

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims

Claims 1-64 (canceled)

Claim 65 (currently amended) A method for detecting protein-protein interaction between a first test polypeptide and a second test polypeptide, comprising:

expressing in a host cell a first fusion protein and a second fusion protein, said first fusion protein having said first test polypeptide and an N-intein, said second fusion protein having said second test polypeptide and a C-intein, wherein at least one of the two fusion proteins has an inactive reporter capable of being converted to an active reporter protein upon trans-splicing through said N-intein and said C-intein; and

detecting said active reporter protein wherein the presence of said active reporter protein indicates said first test polypeptide interacts with said second test polypeptide.

Claim 66 (currently amended) A method for detecting protein-protein interaction between a first test polypeptide and a second test polypeptide, comprising:

expressing in a host cell a first fusion protein and a second fusion protein, said first fusion protein having said first test polypeptide, an N-intein, and a first inactive reporter polypeptide, said second fusion protein having said second test polypeptide, a C-intein, and a second inactive reporter polypeptide, wherein ligation between the C-terminus of said first inactive reporter polypeptide and the N-terminus of said second inactive reporter polypeptide forms an active reporter protein; and

detecting said active reporter protein wherein the presence of said active reporter protein indicates said first test polypeptide interacts with said second test polypeptide.

Claim 67 (previously presented) The method of Claim 66, wherein at least one of said first or second fusion proteins further contain an amino acid sequence capable of enabling at least one of the expressed first or second fusion proteins to at least partially anchor to the cell membrane of the host cell.

Claim 68 (previously presented) The method of Claim 67, wherein the first inactive reporter polypeptide is fused to the N-terminus of the N-intein.

Claim 69 (previously presented) The method of Claim 67, wherein said second inactive reporter is fused to the C-terminus of said C-intein.

Claim 70 (previously presented) The method of Claim 67, wherein said first inactive reporter polypeptide is an N-terminal fragment of said active reporter protein and said second inactive reporter polypeptide is the remaining C-terminal fragment of said active reporter protein.

Claim 71 (previously presented) The method of Claim 67, wherein said host cell is a diploid yeast cell generated by mating a first haploid yeast cell having a first chimeric gene encoding said first fusion protein with a second haploid yeast cell having a second chimeric gene encoding said second fusion protein.

Claim 72 (previously presented) The method of Claim 67, wherein one of said first or second test polypeptides is at least partially anchored to the cell membrane while the other is contained inside the cell.

Claim 73 (previously presented) The method of Claim 67, wherein both of said first and second test polypeptides are at least partially anchored to the cell membrane.

Claim 74 (previously presented) The method of Claim 67, wherein both of said first and second fusion proteins further contain an amino acid sequence capable of enabling

both of the expressed first and second fusion proteins to at least partially anchor to the cell membrane of the host cell, wherein said first and second test polypeptides at least partially reside outside the cell.

Claim 75 (previously presented) The method of Claim 67, wherein both of said first and second fusion proteins further contain an amino acid sequence capable of enabling both of the expressed first and second fusion proteins to at least partially anchor to the cell membrane of the host cell such that said first and second test polypeptides are exposed outside the cell while the inactive reporter polypeptides and the N-intein and C-intein are retained within the cell.

Claim 76 (previously presented) The method of Claim 75, wherein said first inactive reporter polypeptide is an N-terminal fragment of said active reporter protein and said second inactive reporter polypeptide is the remaining C-terminal fragment of said active reporter protein.

Claim 77 (previously presented) The method of Claim 75, wherein said first test polypeptide is fused to the N-terminus of a first transmembrane domain which is fused to the N-terminus of said first inactive reporter polypeptide that is fused to the N-terminus of said N-intein in said first fusion protein.

Claim 78 (previously presented) The method of Claim 75, wherein said second test polypeptide is fused to the N-terminus of a second transmembrane domain which is fused to the N-terminus of said C-intein that is fused to the N-terminus of said second inactive reporter in said second fusion protein.

Claim 79 (currently amended) A method for detecting protein-protein interaction between a first test polypeptide and a second test polypeptide, comprising:

expressing in a host cell a first fusion protein and a second fusion protein, said first fusion protein having a first test polypeptide fused to the N-terminus of a first transmembrane domain which is fused to the N-terminus of a first inactive reporter polypeptide that is fused to the N-terminus of an N-intein, said second fusion protein having a second test polypeptide fused to the N-terminus of a second transmembrane domain which is fused to the N-terminus of a C-intein that is fused to the N-terminus of a second inactive reporter,

wherein when expressed in said host cell said first and second fusion proteins are anchored to the cell membrane of the host cell with said first and second test polypeptides being exposed to the outside the cell, and the inactive reporters, the N-intein and C-intein all being retained within the cell,

wherein ligation between the C-terminus of said first inactive reporter polypeptide and the N-terminus of said second inactive reporter polypeptide forms an active reporter protein,

wherein said host cell lacks said active reporter protein; and
detecting said active reporter protein wherein the presence of said active reporter protein indicates said first test polypeptide interacts with said second test polypeptide.

Claim 80 (currently amended) A method for detecting protein-protein interaction, comprising:

providing a prey fusion protein expression library comprising a plurality of chimeric genes contained in a plurality of prey haploid yeast cells of a first mating type, wherein each of said plurality of chimeric genes encodes a fusion protein containing a prey test polypeptide, an N-intein, a first inactive reporter polypeptide;

providing a plurality of bait haploid yeast cells having a mating type opposite to that of said prey haploid yeast cells, said bait haploid yeast cells expressing a bait fusion protein containing a bait test polypeptide, a C-intein, a second inactive reporter polypeptide, and an amino acid sequence which enables the bait fusion protein to anchor to the cell membrane of the bait haploid yeast cell, wherein ligation between said first

inactive reporter polypeptide and said second inactive reporter polypeptide forms an active reporter protein;

mating said plurality of bait haploid yeast cells and said plurality of prey haploid yeast cells to form a plurality of diploid yeast cells; and

detecting said active reporter protein in said plurality of diploid yeast cells
wherein the presence of said active reporter protein indicates said prey test polypeptide interacts with said bait test polypeptide.

Claim 81 (previously presented) The method of Claim 80, wherein said prey test polypeptide is in the cytoplasm of said diploid yeast cells.

Claim 82 (previously presented) The method of Claim 80, wherein said prey test polypeptide is at least partially exposed outside said diploid yeast cells.

Claim 83 (previously presented) The method of Claim 80, wherein said prey test polypeptide at least partially resides in the cell membrane of said diploid yeast cells.

Claim 84 (currently amended) The method of Claim[[s]] 80, wherein said prey test polypeptide is fused to the N-terminus of a transmembrane domain which is fused to the N-terminus of said first inactive reporter polypeptide that is fused to the N-terminus of said N-intein in said prey fusion protein, and wherein said bait test polypeptide is fused to the N-terminus of a transmembrane domain which is fused to the N-terminus of said C-intein that is fused to the N-terminus of said second inactive reporter in said bait fusion protein.

Claim 85 (currently amended) A method for detecting protein-protein interaction, comprising:

providing a prey fusion protein expression library comprising a plurality of chimeric genes contained in a plurality of prey haploid yeast cells of a first mating type, wherein each of said plurality of chimeric genes encodes a fusion protein containing a prey test polypeptide, a C-intein, a first inactive reporter polypeptide;

providing a plurality of bait haploid yeast cells having a mating type opposite to that of said prey haploid yeast cells, said bait haploid yeast cells expressing a bait fusion protein containing a bait test polypeptide, an N-intein, a second inactive reporter polypeptide, and an amino acid sequence which enables the bait fusion protein to anchor to the cell membrane of the bait haploid yeast cell, wherein ligation between said first inactive reporter polypeptide and said second inactive reporter polypeptide forms an active reporter protein;

mating said plurality of bait haploid yeast cells and said plurality of prey haploid yeast cells to form a plurality of diploid yeast cells; and

detecting said active reporter protein in said plurality of diploid yeast cells wherein the presence of said active reporter protein indicates said prey test polypeptide interacts with said bait test polypeptide.

Claim 86 (previously presented) The method of Claim 85, wherein said prey test polypeptide is in the cytoplasm of said diploid yeast cells.

Claim 87 (previously presented) The method of Claim 85, wherein said prey test polypeptide is at least partially exposed outside said diploid yeast cells.

Claim 88 (previously presented) The method of Claim 85, wherein said prey test polypeptide at least partially resides in the cell membrane of said diploid yeast cells.

Claim 89 (currently amended) The method of Claims 85, wherein said ~~prey~~ bait test polypeptide is fused to the N-terminus of a transmembrane domain which is fused to the N-terminus of said ~~first~~ second inactive reporter polypeptide that is fused to the N-

terminus of said N-intein in said prey fusion protein, and wherein said prey ~~bait~~ test polypeptide is fused to the N-terminus of a transmembrane domain which is fused to the N-terminus of said C-intein that is fused to the N-terminus of said first ~~second~~ inactive reporter in said bait fusion protein.

Claim 90 (previously presented) A kit comprising:

 a first vector containing a first chimeric gene encoding a first inactive reporter polypeptide fused to the N-terminus of an N-intein and containing an operably linked first multiple cloning site (MCS) such that when a nucleic acid encoding a first test polypeptide is inserted into said first multiple cloning site, said first chimeric gene is capable of expressing a first fusion protein containing said N-intein, said first test polypeptide, and said first inactive reporter polypeptide fused to the N-terminus of said N-intein;

 a second vector containing a second chimeric gene encoding a second inactive reporter polypeptide fused to the C-terminus of a C-intein and containing an operably linked second multiple cloning site (MCS) such that when a nucleic acid encoding a second test polypeptide is inserted into said second multiple cloning site, said second chimeric gene is capable of expressing a second fusion protein containing said C-intein, said second test polypeptide, and said second inactive reporter polypeptide fused to the C-terminus of said C-intein, wherein ligation between the C-terminus of said first inactive reporter polypeptide and the N-terminus of said second inactive reporter polypeptide forms an active reporter protein; and

 instructions for using said first and second vectors in detecting protein-protein interactions.

Claim 91 (previously presented) The kit of Claim 90, further comprising a host cell deficient in said active reporter protein.

Claim 92 (previously presented) The kit of Claim 90, wherein at least one of said first and second chimeric genes further contains a nucleotide sequence encoding an

amino acid sequence capable of enabling at least one of the expressed first or second fusion proteins to at least partially anchor to the cell membrane of the host cell.